

## Chronic Allograft Nephropathy: The Mechanisms and Strategies

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Chronic allograft nephropathy (CAN) is the main cause of long-term renal allograft loss. It is an active but slowly progressive injury mainly caused by alloreactivity to the graft, and further deteriorated by non-immunologic nonspecific factors. After an overview of the pathologic characteristics of CAN based on the Banff Working Classification, the underlying mechanisms and therapies are explored in this review, with an emphasis on novel theories and new findings. With regard to the mechanisms, indirect antigen presentation, ICOS-B7h costimulatory pathways, the roles of immune cells, humoral immunity, epithelial-mesenchymal transition and fibrogenesis are paid more attention than non-immunologic factors including calcineurin inhibitor (CNI) nephrotoxicity, ischemia/reperfusion, senescence and other various factors. With regard to therapeutic strategy, we line up the clinical and experimental proceedings based on the mechanisms. Sufficient immunosuppression, CNI sparing, rapamycin conversion, and administration of mycophenolate mofetil have been applied clinically and are discussed. New experimental therapies on deletion of humoral factors, blockade of costimulation pathway, intervention of fibrogenesis, blockade of signal transduction pathway, protection of endothelium and tissues, and induction of accommodation are introduced together with other nonspecific treatments. [*Hong Kong J Nephrol* 2007;9(2):58–69]

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慢性移植腎病嚴重危害了移植物的長期存活。它是由同種免疫反應導致的一種主動但是緩慢進行的病理損害，並為非特異性損傷因素加重。本文首先介紹它以 Banff 標準為依據的病理學特徵，隨後探討發病的機理及應對策略，著重於近年來的新發現和新成果。機理研究方面，側重敘述間接抗原提呈、ICOS-B7h 共刺激通路、免疫細胞功能、體液免疫、上皮間充質轉變和組織纖維化等方面的新進展，同時涉及到鈣調神經素腎毒性、缺血/再灌注損傷、器官老化及其它非特異性損傷因素的作用。應對策略方面，主要探討基於相應發病機理的臨床和基礎研究方面的新舉措。有效的免疫抑制、鈣調神經素撤離、雷帕黴素轉換、嗎替麥考酚酯使用等方法因已獲應用而被首先提及。隨後重點討論新的實驗研究進展：清除體液因素、阻斷共刺激通路、拮抗纖維化、阻斷信號轉導通路、內皮和組織保護以及移植物適應狀態的誘導和相關非特異性因素的處理。希望本文對慢性移植腎病進一步的研究工作起到推動作用。

### INTRODUCTION

Chronic allograft nephropathy (CAN) is the main cause of renal allograft loss. There is a reported prevalence of CAN of 60–70% in protocol biopsies 1 year after transplant [1]. With the increasing number of recipients

and the limited donor pool, transplant researchers have shifted their focus toward the underlying mechanisms of CAN in order to develop effective and viable treatments in both clinical and basic areas to improve long-term outcomes. The etiology of CAN include both immune and non-immune causes. Furthermore, these

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causes interact to make diagnosis and treatment more complicated. Herein, we use this term to mean active but slowly progressive injury caused by alloreactivity to the graft and further deteriorated by non-immunologic nonspecific factors. That means the immunologic damage, so-called chronic rejection, provides the allograft a basic background or platform for CAN. Based on this cognition, we focus on immune-related rather than non-immune-related knowledge in this review. It would be impossible to separate all the individual parts of the mechanisms and strategies due to their overlap; instead, we try to follow the entire process of the immune response to elucidate the mechanisms, with an emphasis on new clinical and experimental strategies.

## WHAT IS CAN?

CAN is usually characterized clinically as chronically deteriorating renal allograft function, often in combination with proteinuria and aggravation or *de novo* hypertension [2]. To diagnose, pathologic evidence is required and somewhat more important than clinical data because individuals suffering from CAN may have stable and even optimal graft function.

The current criteria for CAN are given by the Banff Working Classification of Renal Allograft Pathology, which originated in a meeting held in Banff, Canada on August 2–4, 1991 [3]. The Banff 97 working classification of renal allograft pathology is followed worldwide [4]. Based on this classification, an adequate specimen is defined as a biopsy with 10 or more glomeruli and at least two arteries, with the threshold for a minimal sample of seven glomeruli and one artery. It is also recommended that at least two separate cores containing cortex are obtained, or two separate areas of cortex in the same core are included. Chronic changes in the renal allograft can be seen in the glomeruli, vessels, tubules, and interstitium. For allograft glomerulopathy, the presence of double contours is the most specific change, following the increase of the mesangial matrix. Chronic allograft arteriopathy is characterized by concentric thickening of the intima, sometimes with inflammatory cells infiltrating under the endothelium. The interstitium in CAN shows an increase in fibrosis with corresponding tubular atrophy. Generally, the severity of CAN is graded according to histopathologic findings as grade I (mild), grade II (moderate) or grade III (severe), which is defined by the mild, moderate or severe interstitial fibrosis (ci0 to ci3) and tubular atrophy (ct0 to ct3). CAN grade I requires minor changes (equivalent to ci1, ct1), CAN grade II requires moderate changes (ci2/ct2, ci1/ct2, ci2/ct1), and CAN grade III requires severe changes (ci3/ct3, ci2/ct3, ci3/ct2). Tubulointerstitial and

glomerular damage, once established, are irreversible, resulting in declining renal function and graft failure. The term “irreversible point”, suggested by Tullius et al in a rat CAN model, emphasizes that there are two phases in CAN progression based on clinical and pathologic alterations [5]. In an outstanding 10-year clinical study, two distinctive phases of injury were evident as CAN evolved [6]. An initial phase of early tubulointerstitial damage from ischemic injury, prior to severe rejection and subclinical rejection, predicted mild disease by 1 year. Beyond 1 year, a later phase of CAN was characterized by microvascular and glomerular injury. By 10 years, severe CAN was present in 58.4% of patients, with sclerosis in 37.3% of glomeruli.

The 8<sup>th</sup> Banff conference on allograft pathology was held in 2005, and the updated classification tries to eliminate the diagnostic term “CAN” because it has been used for all causes of chronic renal allograft dysfunction with fibrosis, hindering accurate diagnosis and appropriate therapy. With the recognition of the entity of chronic antibody-mediated rejection and based on new pathologic knowledge, the traditional CAN has been divided into three parts: (1) chronic active antibody-mediated rejection; (2) chronic active T cell-mediated rejection; (3) interstitial fibrosis and tubular atrophy with no evidence of any specific etiology [7]. Again, we can find that these categories depend on the causes of both alloantigen and non-alloantigen. In the clinical setting and even in the animal kidney transplantation setting, these categories are usually present together so that there is still a need for the term “CAN” for easier discussion until a more precise term can be settled on.

## THE UNDERLYING MECHANISM OF CAN

CAN is an active procedure that is closely related to immunoreactivity. The underlying mechanisms are detailed below, according to the sequence of the entire immune response, tissue and cell pathophysiology, and nonspecific factors. All causes lead to damage, followed by fibrogenesis, which are the central characteristics of CAN.

### *Human leukocyte antigen matching*

No doubt, human leukocyte antigen (HLA) matching between donor and recipient has a strong effect on renal allograft long-term survival. In humans, HLA-matched grafts have an estimated half-life of 12.4 years compared with 8.6 years for HLA-mismatched grafts [8]. The well developed cross-reactive group (CREG) matching is associated with a reduced frequency of late acute rejection episodes and improved graft function at 2 years [9], while sharing less CREG is correlated with inferior long-term graft survival [10].

### **Panel reactive antibodies**

Panel reactive antibodies (PRA) represent the overall antibody level against a particular human population in the given individual. A high PRA value in a recipient candidate usually indicates a high possibility of an immune response to a random donor from that population. Although alloantibody level represented by cross-match prior to transplantation could be negative, a high PRA before and after grafting remains a high risk factor for progression of CAN. It has been reported that with the decrease in blood transfusions since the introduction of erythropoietin, there has been a substantial decrease in mean PRA value, resulting in longer-term survival [11]. In his humoral theory of chronic rejection, Terasaki believes that elevated PRA in the recipient after transplantation is one key factor in the progression of CAN [12,13].

### **Antigen presentation**

Antigen presentation to T cells plays a central role in the immune response. Antigen presentation directs the following immunoreactivity model and regulates ongoing chronic rejection. Based on different antigen processing and presenting mechanisms, T cells recognize foreign HLA antigens via direct or indirect pathways. In the direct pathway, donor-derived antigens are presented on donor-derived professional antigen-presenting cells (APCs) to recipient T cells, which mainly results in the activation of CD8+ T cells and thus T cell effector functions. Alternatively, indirect presentation is mediated by uptake of donor antigens by recipient-derived APCs graft-infiltrating and/or in the secondary lymphoid organs. Indirect antigen presentation has been suggested to play an important role in the induction of chronic rejection [14,15]. Indirect presentation might result in activation of B cell responses and thus production of alloantibodies [14, 15]. Furthermore, the indirect pathway might be subtly related to chronic CD4+ T cell-mediated injury to the allografts; among the recognized antigens could be subdominant and/or cryptic epitopes derived from the dominants processed by non-professional APCs or APCs in non-draining lymphoid compartments or from *de novo* exposed antigens by immunologic damage in tissue debris, termed epitope spreading. At a later stage, the immune response diversifies into multiple determinants of the same or other antigens [16,17]. Moreover, non-professional APCs may induce a unique subset of cytotoxic CD8+ T cells (CTL) that are less efficient at cell killing, partly explaining a chronic alloresponse (allograft arteriopathy) to the persistence of alloantigens [18–20].

For full activation and proliferation, T cells need at least two signals: the first signal, which is mediated through the T cell receptor, and the second, co-stimulation, signal, which is transferred through cell

surface receptors by interaction with their ligands (CD28-B7, CD40-CD40L, ICOS-B7h, LFA-1-ICAM-1) [21,22]. The CD28-B7, CD40-CD40L and ICOS-B7h costimulatory pathways are intimately linked, because the IgG switching defect in ICOS knockout mice can be rescued by CD40 signaling and ICOS costimulation has been shown to increase CD40L expression, suggesting that ICOS is upstream of CD40-CD40L in the same pathway [23]. Although the CD28-B7 and CD40-CD40L pathways are thought to be important in acute rejection [24], there is evidence to support their roles in modulating chronic rejection, including CAN, in experimental models. Both CD40-CD40L and ICOS-B7h contribute to CAN [25], but the ICOS-B7h pathway more profoundly responds to CAN. First, B7h is constitutively expressed on B cells and is inducible on monocytes, dendritic cells, fibroblasts, and endothelial cells (ECs) [26,27]. Second, ICOS-B7h interactions are important for the development of effector CD8+ and CD4+ cells [28, 29]. Furthermore, this pathway is shown to modulate not only T-helper 1 (Th1)-dependent response but also Th2 signaling [28,29]. Moreover, the antigen in the context of major histocompatibility complex with the appropriate T cell receptor in the presence of CD40-CD40L and ICOS-B7h costimulation results in activation of B cells and antibody production [30,31]. This pathway is more responsible for the late immune response, memory lymphocyte reaction and chronic rejection [23].

### **Cellular immunity**

There is evidence to show that CD4+ and CD8+ T cells play roles in recipients with CAN. In research on the contribution of CD4+ and CD8+ T cells and interferon- $\gamma$  to CAN in humans, Obata et al found that the Th1 response mediates both acute and chronic rejection [32]. Our data and those of others show that the Th2 response, which directs immune reaction to shift to humoral immunity, may play a more significant role in CAN than Th1 [33,34]. Koch et al proved that adoptive transfer of primed CD4+ T cells induced a pattern of CAN in a nude rat model [35]. Kuo et al provided evidence for the seminal role of CD4+ T cells in the pathogenesis of obliterative airway disease, which represents chronic rejection in the lung graft [36]. It has been postulated that the chronicity of the vascular injury may be due to a delayed-type hypersensitivity response that is intrinsically ineffective in eliminating donor antigens that are located on ECs lining the vessels [37,38]. The activated CTLs can lyse the target cells and result in a tissue lesion or scar which is closely related to the following fibropathogenesis. As mentioned above, the insufficient cell killing of the CTLs could reduce chronic allograft arteriopathy [18–20]. Furthermore,

CD103+CD8+ T cells have the capacity to combine with tubular epithelial cells via their counter-receptor E-cadherin, inducing the process of epithelial-mesenchymal transition (EMT) and, later, CAN [39]. Moreover, infiltrating inflammatory cells (macrophages, natural killer cells, neutrophils) promote the process of CAN by secreting a variety of cytokines and growth factors for local damage [40].

### ***Humoral immunity***

There is accumulating evidence to indicate that humoral immunity plays a role in CAN. C4d is one of the degradation products of complement component C4 that remains covalently linked to the tissue after activation. C4d is thought to be more stably deposited compared to IgG, and it is therefore frequently used as a marker for humoral rejection [41,42]. Glomerular C4d deposits have been found in about 16% of renal allograft biopsies [43], whereas with transplant glomerulopathy, the majority of patients have glomerular C4d deposits [44]. The presence of C4d deposits in allograft biopsies seems to be an independent predictor of CAN [45].

Humoral responses can be directed against HLA or non-HLA antigens of the graft. Renal transplant recipients with anti-HLA antibodies are 5–6 times more likely to develop chronic rejection and lose their grafts [46]. *De novo* anti-HLA antibodies post-transplantation have also been correlated with chronic rejection and the poorest graft outcome [47,48]. In addition, almost all patients with chronic rejection have had circulating antibodies against HLA antigens, but not necessarily donor HLA antigens [49]. Antibodies reactive with mesangial cells and glomerular basement membrane antigens were also found in the development of chronic rejection [50–52]. The antigens involved were identified as the heparan sulfate proteoglycan perlecan and the  $\alpha 1$  chain of collagen type VI in association with the  $\alpha 5$  chain in the glomerular basement membrane [51,52]. Furthermore, antibodies against non-HLA antigens on ECs could result in apoptosis or proliferation, which accelerates the process of CAN [53,54].

### ***Acute rejection/subclinical rejection***

It is clear that there is a close relationship between acute rejection and chronic rejection. Acute rejection episodes within the first 3 months may have no effect on chronic rejection, whereas acute rejections occurring after 6 months confer the greatest risk [55]. Ten-year graft survival rates censored for causes of graft loss other than chronic rejection were 94%, 86%, and 45% for patients without an acute rejection episode, with early acute rejection episodes, and with late acute rejection episodes, respectively [9]. The estimated half-life for cadaveric transplants is shorter in patients who have experienced an acute rejection episode than in those who have not, 6.6 years versus 12.5 years [56].

Compared with acute rejection, subclinical rejection may be more dangerous for patients as the clinical alteration is mild and often undetectable due to the kidney's potent compensation capability. In this case, the mild tubulitis, intimal arteritis and infiltrating lymphocytes lead to slow but persistent damage, inducing fibrogenesis.

### ***Proinflammatory factors, chemokines and growth factors***

It has been shown that proinflammatory mediators, cytokines and multiple chemokines are involved in alloreactivity. Cytokines secreted by lymphocytes, macrophage, and by the cells of inflamed tissue could be responsible for the formation of arteriosclerotic lesions. Leukocyte attraction and trafficking to sites of immune reactions are controlled by chemoattractant cytokines and interleukins. The chronic antigenic stimulation of T cells by graft HLA antigens results in the production of cytokines that damage the vascular endothelium. ECs may then secrete growth factors that induce the proliferation of smooth muscle cells (SMCs) and the migration of myocytes from the media into the intima.

### ***Graft endothelium***

The vascular endothelium is the first place of donor-recipient contact. ECs could be injured and then activated to express more E-selectin, intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 and nuclear factor kappa B, and secrete proinflammatory factors and endothelin (ET). Elevated ET levels are associated with inflammation and immune responses as well as with transplant-associated diseases, contributing to cell activation and growth. ET expression is stimulated by chemokines, and ET itself stimulates the synthesis of chemokines under certain conditions [57]. ECs are also the first target of alloreaction, resulting in changes in morphology and function of ECs and then apoptosis, which participates in CAN progression.

### ***Cell proliferation and fibrogenesis***

Graft vasculopathy is an intimal fibroproliferative process; intimal hyperplasia due to the accumulation of SMCs is a key factor in the pathogenesis. Both immune- and drug-induced vascular lesions are characterized by an inflammatory response that is accompanied by the release of cytokines and growth factors that stimulates SMC migration to the intima. SMCs are then driven by the inflammatory milieu to further activation, leading to proliferation and deposition of extracellular matrix and resulting in intimal thickening, flow obstruction, and tissue ischemia. Most of the proliferating cells in early neo-intimal development are graft-derived SMCs [58,59].



In the late stage, however, the SMCs are host-derived: SMCs or myofibroblasts migrating into the graft from adjacent vessels [60], the progenitor cells released from vasculature and reaching the graft via circulation [61,62], and bone marrow-derived progenitor cells reaching the graft via circulation [63,64]. In the vessels of the kidney suffering from CAN, the pathophysiology is similar [65]. But regarding interstitial fibrosis, the fibrogenetic cells originate from several places: 14–15% are bone marrow-derived fibroblasts, 36% are derived from tubular EMT, and the rest is from the proliferation of native local fibroblasts [66]. During the EMT process, the tubular epithelial cells lose the epithelial cell characteristics, gradually acquire mesenchymal cell characteristics, and finally transdifferentiate toward fibroblasts, resulting in interstitial fibrosis. Activated CD4+ and CD8+ lymphocytes, macrophages or CD103+ cells (a regulatory T cell marker), and even calcineurin inhibitor (CNI) nephrotoxicity, are all likely to be responsible for this transition [33,39,67,68]. Furthermore, some signal transduction pathways, including p38, ERK1/2, STAT, PKC, and E2A [68–71], are involved in EMT. Strutz and Muller have declared that transdifferentiation has come of age [72].

### ***CNI nephrotoxicity***

CNI improves early graft survival significantly. However, the balance between preventing immunologic allograft loss and the management of CNI-related nephrotoxicity is still an issue in renal transplantation. CNI initiates and sustains vascular injury and tissue ischemia, characterized by nodular hyaline deposit on the outer part of the arteriole [4], resulting in acute and chronic effects ranging from vasoconstriction to activation of ECs, significant increase in growth factors and cytokine synthesis and release, inhibition of endothelial nitric oxide production and extracellular matrix accumulation, cell migration, and proliferation [68,73,74].

### ***Brain death and ischemia/reperfusion***

Brain death and ischemia/reperfusion (I/R) may culminate clinically in delayed graft function (DGF), which is defined as a requirement for dialysis during the first week after transplantation, and trigger an inflammatory cascade with upregulation of cytokines, and adhesion and HLA-DR molecules [75,76]. This injury increases graft immunogenicity leading to more early acute rejection episodes. Although fully recovered DGF without acute rejection episodes may not necessarily be detrimental for long-term graft survival [77], according to the UNOS (United Network for Organ Sharing) database of almost 89,000 cadaveric donor transplant recipients, DGF is associated with a small increased risk of CAN [78].

### ***Senescence***

Cells in senescence show alterations in shape, expression of extracellular matrix metalloproteinases (MMP) and cytoskeletal collagens with several characteristics including shortened telomeres, increased expression of specific tumor suppressor genes, and increased activity of senescence-associated  $\beta$ -galactosidase (SA $\beta$ -gal) [79]. The cell cycle inhibitors, p16 and p21, have been studied in relation to cellular senescence, and both are thought to be involved in G1 arrest [80,81]. In this case, the biological age of the chronically rejected kidney is higher than the chronological age [80,81].

### ***Other non-immunologic factors***

Many other factors could have effects on CAN progression, including cadaveric donor, old donor age, renal insufficiency, cytomegalovirus infection, overweight, recipient smoking, non-compliance, hypertension, proteinuria, hyperlipidemia, diabetes and so on. Moreover, young age is also a risk factor that is associated with a relatively high state of immune responsiveness to alloantigens.

## **THE STRATEGIES: WHAT WE CAN DO**

Even if the contributing factors to CAN can be identified, not all of them can be interrupted prior to and after grafting. In many settings, transplant candidates have no better choice for donor HLA typing, cadaveric, old age, and renal insufficiency. After operation, these factors are not amenable to therapy. Indeed, clinicians can only do what they can. In the section below, we focus on the practical clinical and experimental strategies based on the corresponding mechanisms.

### ***Immunologic matching***

In theory, the ideal donor is one with an identical immunologic background as the candidate. In reality, this will always be limited by the donor pool. Realistic requirements are a negative cross-match and a HLA/CREG mismatch that is as low as possible. If the PRA value of the candidate is higher than expected, some preconditioning treatment such as tacrolimus/mycophenolate mofetil (MMF) administration, high-dose intravenous immunoglobulin (IVIg) and immunoabsorption is helpful, not only for acute rejection but also later for CAN.

### ***Sufficient administration of immunosuppressant against acute rejection/subclinical rejection***

The extent of the alloresponse is a balance between the immunogenicity of the graft, recipient responsiveness, and the level of immunosuppression.

After transplantation, a sufficient level of immunosuppression is definitely required to prevent the onset of acute rejection that may destroy both the function and the structure of nephrons and generate a high risk for CAN. But in some clinical settings, including consideration of the side effects of immunosuppression, DGF, and economic problems, the serum level of immunosuppressant is relatively low (although the value is within the general therapeutic window), which is the main cause of the initiation of subclinical rejection. It is tempting for the patient and clinician to ignore the underlying progressive damage of the mild activated immune reaction due to a satisfactory serum creatinine level. In a small-scale prospective clinical study, up to 50% of allograft kidney biopsy samples were found to have mild acute rejection with lymphocyte infiltration; high-dose methylprednisolone bolus treatment for 3–5 days could often stabilize kidney function and, in some cases, decrease creatinine level to some extent [67]. A sufficient level of immunosuppression is crucial in preventing CAN; it can be easily achieved, but perhaps we just need to pay more attention to it.

### ***CNI sparing and rapamycin conversion***

Although in some recipients on cyclosporine, conversion to tacrolimus (a potent agent that suppresses both cellular and humoral immunity) resulted in sustained improvement in renal function [67], CNI sparing strategies are thought to be of benefit for CAN patients. Three main protocols have been investigated: CNI minimization, CNI withdrawal, and complete avoidance of CNI [82]. In regimens based on rapamycin, CNI withdrawal is a safe strategy, achieving an improvement in renal function, histology, and graft survival. Rapamycin may have the ability to reduce the rates of chronic rejection by further reduction of the incidence of acute rejection episodes, and inhibit the proliferation of SMCs and fibroblasts to reduce intimal hyperplasia in immune and non-immune models of vascular injury. Rapamycin introduction dramatically reduced intragraft  $\alpha$ -smooth muscle actin expression, whereas reduction in CNI doses resulted in a significant decrease of this marker of fibroblast activation in the 2-year biopsy [83]. *In vitro* studies showed that rapamycin, compared with cyclosporine, tacrolimus, mycophenolic acid, and deoxyspergualin, is the only immunosuppressant with pronounced inhibitory effects on growth factor-stimulated SMC proliferation [84]. In addition, manifestations of chronic rejection are also inhibited by the novel antiproliferative macrolide everolimus (a rapamycin derivative) in preclinical models [85].

### ***Administration of MMF***

MMF can inhibit the proliferation of T cells and B cells [86]. The effects of this drug on T cells are not via the

IL-2 pathway or transduction signals but via inhibition of downregulation of p27<sup>kip1</sup> [87]. MMF inhibits the mixed lymphocyte reaction response to alloantigen *in vitro* [86] and inhibits induction of CTLs and allogeneic rejection *in vivo* [88]. MMF probably mainly inhibits the early stage of proliferation and differentiation of B cells rather than generation of antibodies by plasma cells, a long-term benefit for CAN [89]. It also inhibits dendritic cell maturation and antigen presenting [90]. Besides stabilizing ECs, inhibiting recruitment of mononuclear cells to the allograft, and decreasing nitric oxide and oxidant induced by cytokines, MMF interestingly inhibits the proliferation of SMCs without fibrogenesis, which directly attenuates the graft arteriopathy of CAN [91–93].

### ***Deletion of humoral factors***

The circulating antibodies against HLA and non-HLA (glomerular basement membrane antigens, EC antigens) initiate/aggravate the kidney damage of CAN. To date, there are several choices to remove the circulating antibodies: plasmapheresis (PP), high-dose IVIg, immunoabsorption, splenectomy, and anti-CD20 antibody application. PP, IVIg and immunoabsorption can decrease antibody titer directly. Splenectomy and anti-CD20 antibody application can reduce B cell mass and incapacitate immune surveillance and antigen presentation to downregulate the antibody titer indirectly. But for reasons of economy and convenience, short-term and high-dose IVIg administration may represent the best choice clinically.

### ***Blockade of costimulation pathway***

Blockade of various costimulation pathways have been used in order to prevent both acute and chronic rejection. Both CTLA4-Ig and blocking anti-CD28 monoclonal antibodies have been found to prevent the development of chronic rejection [94,95]. Late treatment of recipients with CTLA4-Ig to block CD28-B7 interactions also attenuated antibody responses [95]. But florid chronic rejection lesions have been reported in long-term surviving cardiac allografts after the blockade of the CD40-CD40L pathway alone or in combination with CD28-B7 blockade [96–99]. Guillot et al found that prolonged blockade of CD40-CD40L interactions by gene transfer of CD40Ig resulted in long-term allograft survival and donor-specific hyporesponsiveness, but did not prevent chronic rejection [98]. Meanwhile, there has been renewed interest in the ICOS-B7h pathway. In nonhuman primates, blockade of CD40L did not affect the production of anti-donor antibodies, but blockade of B7h-ICOS costimulation demonstrated a key role in the prevention of chronic rejection [100]. In addition, decreased anti-donor alloantibodies have been found upon ICOS blockade [101]. The CD40Ig+ anti-ICOS group showed significant decreased graft

infiltration, decreased anti-donor CTL activity, and decreased alloantibodies compared with the CD40Ig-treated group [25].

### ***Treatment against proinflammatory factors, chemokines and growth factors***

Proinflammatory factors and chemokines are generated by lymphocytes and damaged graft tissues. Meanwhile, the local immunologic milieu attracts activated immune cells, including T cells, B cells and macrophages, and results in more severe lesion and the later CAN. Neto et al demonstrated that carbon monoxide inhibited the progression of fibrogenesis and the deterioration of renal allograft function of established CAN via its anti-inflammatory function [102]. In a rat CAN model, Yang et al identified that targeting of macrophage activity by adenovirus-mediated intragraft overexpression of TNFRp55-Ig, IL-12p40 and vIL-10 could ameliorate adenovirus-mediated chronic graft injury, whereas stimulation of macrophages by overexpression of interferon- $\gamma$  accelerated the injury [103]. Keane et al found that IL-13 gene expression was markedly elevated within allografts and associated with macrophage infiltration and activation in a profibrogenic milieu prior to developing fibrosis [104]. Schnickel et al found that a combination of CXCR3 and CCR5 blockade holds great promise in the control of chronic rejection and markedly decreased intimal thickening in a rat heterotopic heart transplantation model [105]. Inhibition of various growth factors is also effective in the treatment of CAN. Daniel et al demonstrated the antiangiogenic function of A20 in ECs via blockade of vascular endothelial growth factor (VEGF)-mediated phosphorylation of PKC $\beta$ II and ERK1/2, and suggested that antiapoptotic and anti-inflammatory effectors could make A20-based therapies ideal for the treatment and prevention of transplant arteriosclerosis [106]. Moreover, Nykanen et al identified VEGF-driven interplay of inflammation and primitive capillary angiogenesis in chronic rejection to control pathologic microvascular and arterial remodeling in cardiac allografts [107]. Recently, our team found that the migration and proliferation of SMCs could be inhibited after downregulation of platelet derived growth factor (PDGF)-BB by Adanti-ERK gene transfer (data not published). We also found that administration of high-dose aspirin could decrease PDGF expression in the vascular allograft so as to ameliorate allograft arteriopathy (data not published). Herrero-Fresneda et al found that hepatocyte growth factor gene electrotransfer attenuated renal allograft scarring by interfering with the profibrotic inflammatory mechanism [108]. In summary, regulation of the proliferation, migration and secretion of fibrogenic cells by cytokine and growth factor blockade is an effective option to treat CAN.

### ***Signal transduction pathway blockade***

Healy et al proved that EMT was accompanied by sustained activation of p38 and p42/44 (ERK1/2) kinase, which are typically associated with a proliferative state [69]. Li et al also demonstrated that advanced glycation end products induced tubular EMT through the ERK1/2 kinase signaling pathway [109]. Our group found that antisense ERK1/2 oligodeoxynucleotide gene therapy could attenuate graft arteriosclerosis of aortic transplant in a rat model [110]. We also observed that adenovirus-mediated antisense-ERK2 gene therapy attenuated CAN [111]. The treatment downregulated the expression of related genes (IL-2, ICAM-1), lessened inflammation, and improved graft histology/function. Whether the interruption of the ERK1/2 pathway protects allograft via inhibition of EMT and fibrogenesis requires further investigation. In an *ex vivo* study, Slattery et al demonstrated that overexpression of E2A proteins could induce EMT in human renal proximal tubular epithelial cells, suggesting a potential role in renal fibrosis [68]. To date, the number of investigations on signal transduction pathway blockade against CAN is low, and many more activities remain for further study.

### ***Protection of endothelium and tissues***

Maintaining a stable endothelium could be a helpful strategy. ET receptor blockade induced pronounced inhibition in circulating levels of IL-1ra and TNF- $\alpha$  and changes in gene expression of IL-1ra, IL-10 and IL-6 in tissue-specific patterns that occurred independently of the development of graft arteriosclerosis [57]. Antioxidative stress treatment is also an alternative. Djamali et al found in the CAN tissues tubular O $_2^{\cdot-}$ , endothelial nitric oxide synthase (NOS) and inducible NOS, and interstitial collagen I, III and O $_2^{\cdot-}$  levels were significantly increased in CAN-associated EMT [112]. They also demonstrated that oxidative stress could be a unifying injury pathway in experimental and human CAN, and graft infiltration macrophages might be an important source of reactive oxygen species through the NADPH oxidase pathway [113].

### ***Other therapies against fibrogenesis***

Because fibrogenesis is a central mechanism in the progression of CAN, all treatments, from prevention of acute rejection to antioxidant therapy, basically aim to attenuate damage and ameliorate fibrosis remodeling. The MMP family has complex roles in CAN, according to the different members. MMP-2 and MMP-9 can degrade type IV collagen to enhance CAN progression [114,115]. MMP-3 promotes SMC migration to the intima [116]. Local activity of MMP-2 can be sufficient to initiate EMT, possibly through a mechanism involving local proteolytic activation of transforming



growth factor- $\beta$  [117]. So, MMP-based therapy might be valuable for CAN. Bone marrow protein-7 can also inhibit EMT via the transforming growth factor- $\beta$  pathway [118], so it might also be a candidate strategy.

### Induction of accommodation

The term accommodation is more commonly used in xenotransplantation than in allotransplantation. But this phenomenon does exist in kidney allografts. Accommodation is when the graft survives well even in the presence of anti-graft antibodies, which indicates that the graft is protecting itself by some subtle mechanisms: elevated expression of antiapoptotic factors or complement regulatory factors. In human renal acute rejection, high expression of bcx-xL and upregulation of Duffy antigen receptor have been identified [119,120]. In allograft with CAN, the complement regulatory factor protectin (CD59) and decay accelerating factor (CD55) were also found to contribute to accommodation and to the attenuation of damage [121]. Semiletova et al introduced allochimeric RT1.A<sup>a</sup> class I major histocompatibility extracts in a cardiac allograft chronic rejection setting and found that the treatment altered humoral immunity and induced vascular accommodation according to the improved level of Bcl-2/Bcl-xL gene expression [122]. We found that overexpression of HO-1 gene could markedly protect rat kidney allograft against CAN at 3 months and 6 months after grafting [33]. At present, research on the effects of antiapoptotic factors or complement regulatory factors to protect allografts from CAN is insufficient and may be the next focus.

### OTHER INTERVENTIONS

Non-immunologic measures to halt or retard the progression of CAN have focused on aggressive control of blood pressure, proteinuria, hyperlipidemia and diabetes. Treatment of hypertension with calcium channel blockers, beta-blockers and angiotensin-converting enzyme inhibitors (ACEI) have similar antihypertensive efficacy after renal transplantation and are often used in combination to achieve adequate control. Significant reduction in proteinuria has been reported as a beneficial effect of ACEI and angiotensin II receptor antagonists in clinical transplantation. Patients taking ACEIs enjoy less severe CAN overall and longer graft survival [123]. Frequently, statins are used to treat hyperlipidemia, especially in patients taking rapamycin. After transplantation, some patients may suffer from diabetes due to the immunosuppressant (especially tacrolimus). In this situation, glucose control or medication conversion should be considered. Anti-virus treatment, diet, weight control, no smoking and good compliance are suggested in certain settings.

Drugs that improve the microcirculation are also helpful. In addition, molecule interruption against shortened telomeres, SA $\beta$ -gal, p16 and p21 could be designed for the senescent allograft in future.

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